

Listing of Claims

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1. (currently amended) A method for determining the amount of pyruvate dehydrogenase (PDH) complex in a biological sample comprising:

- a) contacting a sample comprising PDH complex with an isolated antibody that specifically binds to PDH complex under conditions to allow specific binding of the antibody to solubilized PDH complex present in the sample to form an immunocomplex;
- b) separating remaining sample contents from the immunocomplex; and
- e) detecting the amount of the PDH complex in the separated immunocomplex, thereby determining the amount of the PDH complex in the ~~patient~~biological sample.

2. (currently amended) The method of claim 1, wherein the antibody is an anti-E2 specific antibody, a monoclonal antibody, or a monoclonal anti-E2 specific antibody.

3. – 5. (canceled)

6. (original) The method of claim 1, wherein the PDH complex in the immunocomplex retains PDH activity.

7. (original) The method of claim 1, wherein the antibody is attached to a solid support and the separating includes separating unbound sample contents from the solid support.

8. (currently amended) The method of claim 7, wherein the separating ~~in b)~~ comprises:

- (i) releasing the immunocomplex complex; and
- (ii) separating the immunocomplex from other components of the sample using SDS-PAGE.

9. (currently amended) The method of claim 7, wherein the detecting ~~in e)~~ comprises contacting immunocomplexed PDH complex with a detectable marker that binds

specifically to the immunocomplexed PDH and measuring the amount of detectable marker present on the solid support.

10. (original) The method of claim 7, wherein the solid support is a microtiter plate or beads.

11. and 12. (canceled)

13. (currently amended) The method of claim 1 further comprising:
(i) quantifying the immunocaptured PDH complex detected in the sample by comparing with a standard reference curve obtained using a purified sample of PDH complex;
(ii) determining specific activity of the immunocaptured PDH complex; or
(iii) both (i) and (ii).

14. (canceled)

15. (currently amended) The method of claim 14 13, wherein the sample is obtained from a patient sample and wherein the method further comprises distinguishing between a defect in PDH complex turnover rate and a defect in production of PDH complex in the patient.

16. (currently amended) A method for measuring activity of PDH complex in a sample, said method comprising:

- a) contacting a sample comprising PDH complex with an isolated antibody that specifically bind to PDH complex ~~with~~ under conditions to allow formation of an immunocomplex of the antibody and the PDH complex present in the sample;
- b) contacting the immunocomplex with a reaction mixture comprising a non-limiting amount of one or more substrates necessary for activity of the PDH complex; and
- c) detecting:
(i) the amount of NADH produced in the reaction mixture, wherein the amount of NADH produced indicates the active state of the PDH complex; or

_____ (ii) the level of phosphorylation of immunocomplexed PDH complex in the in the sample as compared with that of an unphosphorylated PDH complex standard, wherein a level of phosphorylation greater than that in the standard indicates a lowered level of activity, and a level of phosphorylation substantially equal to that of the PDH complex in the sample indicates a normal level of activity of the PDH complex in the sample.

17. (canceled)

18. (original) The method of claim 16, wherein the substrates are β -NAD⁺, Coenzyme A, FAD⁺, cysteine, pyruvate, and thiamine pyrophosphate (TPP).

19. (currently amended) The method of claim 16, wherein the detecting ~~in e)~~ comprises:

(i) transferring an electron from reduced NADH to an electron acceptor molecule to produce NADH; and

(ii) determining a change indicating transfer of an electron to the electron acceptor molecule, wherein magnitude of the change indicates biological activity of the PDH complex.

20. (currently amended) The method of claim 19, wherein the electron acceptor molecule is an electron acceptor dye molecule; and ~~the wherein~~ determining a change indicating transfer of an electron in (ii) involves monitoring the reaction mixture spectrophotometrically to ~~determine-detect~~ a change in absorbance of the electron acceptor dye molecule; wherein magnitude of the change indicates biological activity of the PDH complex as compared to that of a comparable healthy sample of PDH complex.

21. (original) The method of claim 20, wherein the electron acceptor dye molecule is selected from diaphorase, resazurin, and a combination thereof.

22. (original) The method of claim 20, wherein the monitoring comprises detecting a change in fluorescence from the dye molecule.

23. (currently amended) The method of claim 20, wherein the detecting ~~in e)~~ comprises:

(i) contacting the reaction mixture with a PDH inhibitor and comparing an amount of resultant inhibition of the PDH complex compared to that of a comparable healthy sample of PDH complex, or

(ii) contacting the reaction mixture with a PDH complex activator and comparing an amount of resultant activation of the PDH complex compared to that of a comparable healthy sample of PDH complex.

24. (currently amended) The method of claim 23(i), wherein the PDH complex inhibitor is selected from sodium arsenite and ATP.

25. (canceled)

26. (currently amended) The method of claim ~~25~~23(ii), wherein the activator is dichloroacetate.

27. (currently amended) A kit for ~~assaying PDH complex activity in a sample~~ use in the method of claim 16, the kit comprising an antibody specific for said PDH complex

28. (canceled)

29. (currently amended) ~~A The method for determining the level of activity in PDH complex in a sample, said method of claim 16, further~~ comprising:

a) ~~contacting an isolated antibody that specifically binds to PDH complex with a sample comprising PDH complex under conditions that allow specific binding of the antibody to PDH complex present in the sample to form an immunocomplex;~~

b) ~~contacting the immunocomplex with a reaction mixture comprising a non-limiting amount of one or more substrates necessary for activity of the PDH complex;~~

e) ~~separating remaining sample contents from the immunocomplex; and~~

d) ~~_____ prior to detecting the level of phosphorylation of immunocomplexed PDH complex, in the in the sample as compared with that of an unphosphorylated PDH complex standard, wherein a level of phosphorylation greater than that in the standard indicates a lowered level of activity, and a level of phosphorylation substantially equal to that of the PDH complex in the sample indicates a normal level of activity of the PDH complex in the sample.~~

30. (original) The method of claim 29, wherein the level of phosphorylation is compared by measuring an amount of negative isoelectric point shift of the immunocomplexed PDH complex compared to the isoelectric point of the standard, the amount of negative isoelectric point shift being directly proportional to the amount of phosphorylation of the PDH complex in the sample.

31. (original) The method of claim 30, wherein the sample is derived from a patient and wherein the amount of negative isoelectric shift is used to screen the patient for a disorder of PDH complex activity.

32. (original) The method of claim 31, wherein the disorder is a disorder of energy production or utilization.

33. (original) The method of claim 32, wherein the disorder is diabetes.

34. (currently amended) A method for screening to detect an active agent that modifies inhibitor or activator activity of a known inhibitor or activator of PDH complex comprising:

- a) contacting a sample containing PDH complex in the presence of a known inhibitor or activator and a test active agent with a PDH complex immunoprecipitating antibody under conditions that allow formation of an antibody/PDH complex immunocomplex; and
- b) determining the degree to which the test active agent modifies the inhibitor or activator activity of the known inhibitor or activator in the sample as compared to inhibitor or activator activity of the known inhibitor or activator in the absence of the test active agent,

thereby detecting an active agent that ~~modified~~modifies inhibitor or activator activity of a known inhibitor or activator of PDH complex.

35. (canceled)

36. (currently amended) The method of claim 34, wherein:

(i) the PDH complex inhibitor is sodium arsenite or ATP and the test active agent decreases inhibitor activity of the PDH complex inhibitor; or

(ii) the PDH complex activator is dichloroacetate and the test active agent decreases activator activity of the PDH complex activator.

37. (canceled)

38. (currently amended) The method of claim 34, wherein the antibody is an anti-E2 specific antibody, a monoclonal antibody, or a monoclonal anti-E2 specific antibody.

39. and 40. (canceled)

41. (original) A method for screening patients to identify patients suspected of having a late onset mitochondrial disorder, said method comprising:

- a) contacting isolated antibodies that immunoprecipitate PDH complex with a patient sample comprising solubilized PDH complex so that the antibodies bind to solubilized PDH complex present in the sample to form an immunocomplex;
- b) separating the immunocomplex from the remaining sample contents; and
- c) detecting a decrease in the amount of PDH complex as compared with an amount in a corresponding normal sample, wherein the decrease indicates the patient is suspected of having the late onset mitochondrial disorder.

42. (original) The method of claim 41, wherein the late onset mitochondrial disorder is selected from late onset diabetes, Huntington's, Parkinson's and Alzheimer's diseases, ALS (amyotrophic lateral sclerosis), and Schizophrenia.

43. (currently amended) The method of claim 41, wherein the separating ~~in b)~~
comprises:

- a~~i~~) releasing the immunocomplex; and
- b~~ii~~) separating the immunocomplex from other components of the sample using SDS-
PAGE.

44. (original) The method of claim 41, wherein the anti-PDH complex antibodies are
attached to a solid support and the antibodies are tagged with a detectable marker.

45. (currently amended) The method of claim 44, wherein the detecting ~~in e)~~
comprises:

- (i) contacting the immunocomplex with a detectable marker that binds specifically to the
immunocomplex and measuring the amount of signal from the detectable marker present on the
solid support, or
- (ii) high throughput screening.

46. (currently amended) The method of claim 44, wherein the solid support is beads
or a microtiter plate.

47. and 48. (canceled)